BIODIVERSITY OF *FUSARIUM OXYSPORUM* (SCHLECHT.) SNYD. ET HANS. ISOLATES IN THE REPUBLIC OF MOLDOVA

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Abstract: The purpose of the research was to study: i) the biodiversity of *Fusarium oxysporum* isolates on the territory of the Republic of Moldova; ii) the peculiarities of the interaction between the host plant (common wheat) and the *F. oxysporum* isolate.The following were used as research material: i) 40 isolates of the variety *F. oxysporum* var. *orthoceras* (*F. o.o*), collected in different localities of the Republic of Moldova. ii) three genotypes of common winter wheat. The research was carried out in laboratory conditions. The obtained data were processed using modern statistical methods (cluster analysis, factor analysis) in the STATISTICA 7 software package. It was found that the *F. o.o* isolates collected exhibit a pronounced morphological polymorphism in terms of mycelium color and density, colony shape, radial growth rate of colonies under optimal temperature conditions (24°C) and thermal alternation 24/9/24°C, 24/35/24°C. Diversity based on the mentioned characters was also found in the isolates from the same locality.
Treating wheat grains with culture filtrates of *F. o.o* isolates and cultivating seedlings at different temperature conditions (22-23°C), 22-23/8-9/22-23°C demonstrated the manifestation of different virulence phenotypes that did not depend on the reaction of the isolates to low or high temperature *in vitro*, but on the specificity of interaction with the host plant, which was reflected in the cluster organization of the isolates.

Keywords: wheat, *Fusarium oxysporum*, isolates, diversity.

INTRODUCTION

Wheat plays a key role in the development of the global economy. As the population continues to grow, wheat production will need to increase significantly in sufficient quantities to provide a necessary supply of the grain as a reliable and sustainable food source. Since covering more land areas with wheat crops is not a sure way, future wheat varieties should have constant or higher yields, and be able to resist and/or tolerate biotic and abiotic stresses resulting from climate change (Nelson *et al.*, 2010).

Root rot in cereal crops, including common wheat (*Triticum aestivum* L.) is a major problem worldwide.

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Is a complex disease, caused by different pathogens in different geographical areas or different pathogens in the succession of ontogenetic stages of plants. The disease has a specific etiology – the participation of a complex of different fungi from different genera and species. Among the most frequently identified are Fusarium species. Due to their high metabolic activity and adaptability, Fusarium fungi are found in various geographical areas. Their wide or narrow distribution depends on the biological characteristics of the species, the climatic conditions of the region and meteorological fluctuations. It is considered that the specie *Fusarium oxysporum* is widespread throughout the planet (Zhang *et al.*, 2006; Michielse, Rep, 2009). In the Republic of Moldova, *Fusarium oxysporum* var. *orthoceras* are the more widespread variety (Lupascu, 2020).

The aim of the present research was to establish the diversity characteristics of *F. oxysporum* var. *orthoceras* isolates from common winter wheat based on morphological and virulence characters.

MATERIAL AND METHODS

The research was carried out under laboratory conditions. In order to identify the peculiarities of morphological variability of *F. oxysporum* var. *orthoceras* isolates, collected in the central and southern areas of the Republic of Moldova (7 localities), 40 fungal isolates extracted from diseased stems of common wheat were investigated (morphology, speed of growth). The towns are: village Brăviceni, v. Mitoc (Orhei district), Chisinau city (2 varieties: Capriana, Viara), v. Bacioi (Ialoveni district), Leova district, v. Cotesti (Cantemir district), v. Doina (Cahul district).

The isolates were coded depending on the locality as follows: Brăviceni – Br1, Br2, Br3, Br4, Br5; Mitoc – M1, M2, M3, M4, M5; Chisinau (Capriana var.) – ChC1, ChC2, ChC3, ChC4, ChC5; Chisinau (Viara var.) – ChV1, ChV2, ChV3, ChV4, ChV5; Bacioi – B1, B2, B3, B4, B5; Leova – L1, L2, L3, L4, L5; Gotesti – G1, G2, G3, G4, G5; Doina – D1, D2, D3, D4, D5.

F. oxysporum isolates are considered to have maximum radial growth at temperatures between 25 and 28°C (Webb, Brenner, Jacobsen, 2015).

Testing the growth capacity of fungal colonies was carried out in 3 variants: w 1) constant temperature of 24°C for 8 days; 2) thermal alternation: 4 days – 24°C, 2 days – 9°C, 2 days – 24°C; 3) thermal alternation: 4 days – 24°C, 2 days – 35°C, 2 days – 24°C. The colony diameter was recorded on days 4, 6, 8 of fungal growth.

As research material, 3 genotypes of common winter wheat served – L S/BT/S, L Sel./Accent, Moldova 16 and 9 isolates of *F. oxysporum* from 3 different localities – Br1, Br2, Br3 (Brăviceni commune), ChV1, ChV3, ChV5 (Chișinau city), L2, L3, L5 (Leova city). *F. oxysporum* var. *orthoceras* isolates were selected according to the principle of the difference in geographical areas from which they were sampled and the ability to grow at low (9°C) and high (35°C) temperature *in vitro* conditions. Culture filtrates (CF) were prepared on the basis of Cszapek liquid nutrient medium.

After inoculation with fungal mycelium, Cszapek medium was maintained for 21 days at 24°C. Wheat grains were kept for 18 hours in CF after which they were rinsed 2 times with distilled water and placed on filter paper moistened with distilled water in Petri dishes, 30 grains in each.

After 6 days of cultivation at 18–19°C (variant I), the seedlings were subjected to morphometric measurements (germination, %; radicle length, mm; seedling length, cm). The vigor index was calculated based on the formula: *germination (%) × seedling length (cm).*

In the variant II, the seedlings were maintained for 2 days at a temperature of 18–19°C, and on day 3 of growth, they were transferred for 8 hours to a temperature of 8–9°C, after which the initial temperature was returned until day 6.

The experiment was carried out in 3 repetitions. The data were statistically processed according to variance, clusterian (dendrograms – agglomerative-iterative method), factorial analysis in the STATISTICA 7 software package.

RESULTS AND DISCUSSION

The polymorphism in *F. oxysporum* is crucial for understanding its pathogenicity, host range, and for developing strategies to manage diseases caused by this fungus. In essence, the polymorphism in *F. oxysporum* is a key factor in its ability to cause disease in a wide array of plants and to adapt to different environments. Understanding this genetic diversity is essential for developing effective disease management strategies [https://www.google].

Our observations found that *F. oxysporum* var. *orthoceras* isolates differ from each other even when extracted from the same host plant (Fig. 1).

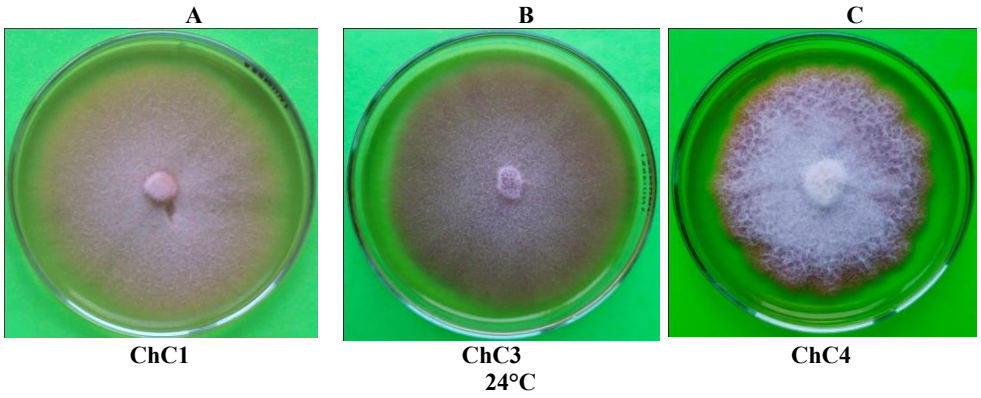


Figure 1. Colonies of *F. oxysporum* var. *orthoceras* isolates (Căpriana variety) at 24°C.

Isolates from the common wheat variety Căpriana (Chisinau) were distinguished by density, mycelium color, and colony edge. ChC2 had a straight, uniform edge, ChC1 had a slightly scalloped edge, and ChC4 had a more pronounced scalloped edge.

Although there is some understanding of the genetic and phenotypic diversity of the fungus *F. oxysporum*, including the existence of pathogenic and nonpathogenic isolates and variable levels of resistance among soybean cultivars (Ellis *et al.* 2014; Lanubile *et al.*, 2015), the effects of temperature and pH in the plant – *F. oxysporum* interaction are unknown.

Under the influence of the temperature 24/9/24°C, dense mycelial rings were formed that were morphologically distinct in the 3 isolates under study (Fig. 2).

At 24/35/24°C, ChC1 and ChC3 formed concentric lilac-colored rings of varying intensity, as well as scalloped colony edges. In ChC4, the scalloped colony edges were not observed, which was recorded at 24°C and 24/9/24°C, but the mycelium formed a wide and dense ring on days when the temperature was 35°C. It is interesting that at both thermal alternations, the dense mycelium ring formed in one and the same isolate – ChC4, although its width was different (Fig. 2).

In relation to what was observed, we mention the opinion of the authors Ritz, Crawford (1999): "by virtue of the capacity for dynamic pleomorphism, the morphological characters of growing fungal colonies can change depending on environmental conditions" and Prosser (1993):

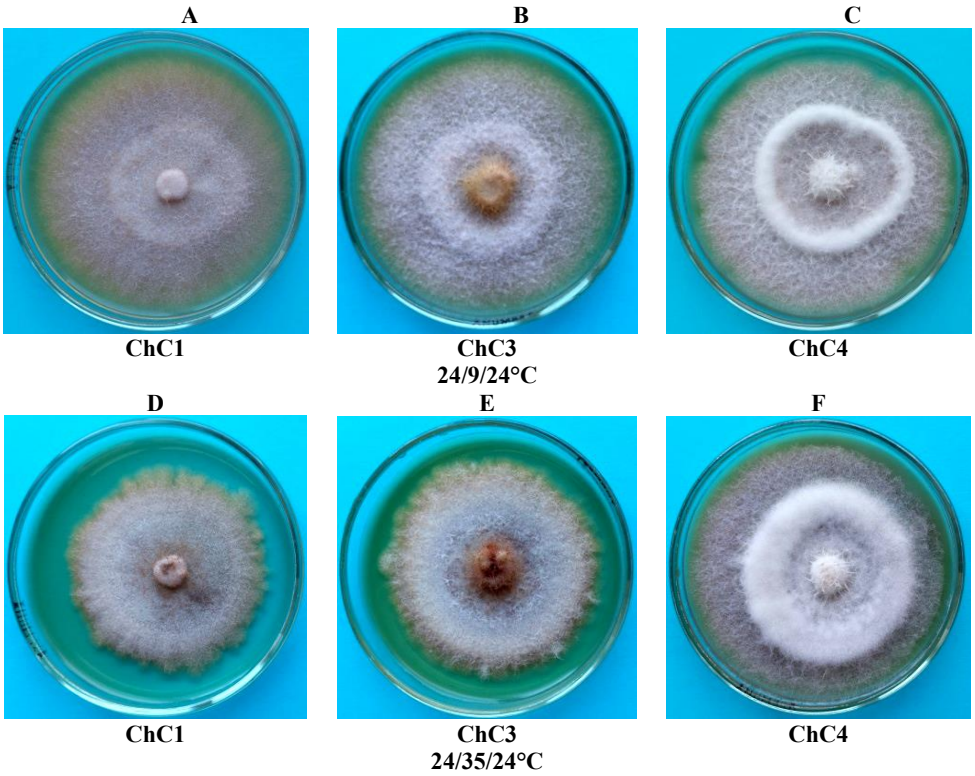


Figure 2. Colonies of F. oxysporum var. orthoceras isolates (Căpriana variety) under the influence of thermal alternations.

"environmental factors are determinants of the colony branching pattern, more efficient for the use of the nutrient substrate in case of minimal synthesis of biological mass".

The study of the reverse of the colonies found that unfavorable thermal action (9 and 35°C) influenced the chromatic profile of the colonies, namely by inhibiting pigmentogenesis (Fig. 3).

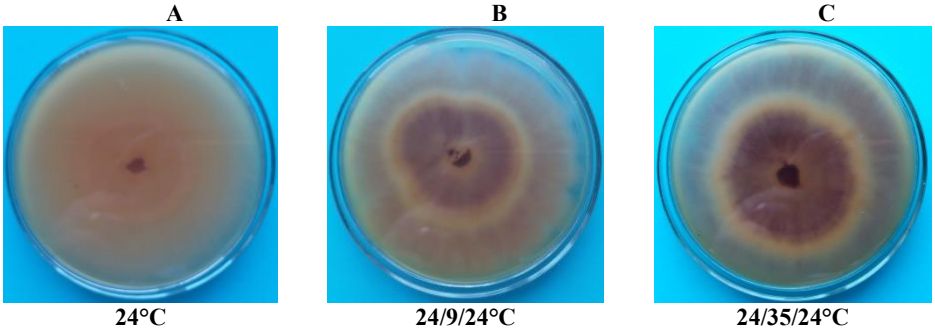


Figure 3. Revers of the *F. oxysporum* var. *orthoceras* colonies (ChC4 isolate).

A relatively high dependence (0.68*, $p<0.05$) was found between the radial growth capacity of *F. oxysporum* var. *orthoceras* isolates at 24°C and 24/9/24°C, and medium (0.38*, $p<0.05$) – between 24°C and 24/35/24°C, which denotes the more pronounced reaction specificity of the isolates at high temperature.

The calculation of the average diameter of the colonies for 5 isolates of each wheat sample demonstrated that the ratio of the diameter in the last and recording – day 8 in relation to day 4 (first measurement) at constant temperature 24°C varied within the limits of 183.0–206.8%; at the 24/9/24°C alternation: 152.2–165.3%, and at the 24/35/24°C alternation: 133.8–185.7%. The data demonstrate that temperatures of 9 and 35°C significantly inhibited radial growth of under study *F. oxysporum* var. *orthoceras* isolates (Table 1).

Table 1

Influence of temperature on the *in vitro* growth of *F. oxysporum* var. *orthoceras* isolates, extracted from common wheat plants in the central and southern localities of the Republic of Moldova

Braviceni v. (Orhei)												
Isolate *F. o.o.*	24°C				24/9/24°C				24/35/24°C			
	I, mm	II, mm	III, mm	III/I, %	I, mm	II, mm	III, mm	III/I, %	I, mm	II, mm	III, mm	III/I, %
Br1	43	69	90	209.3	45	50	68	151.1	43.5	53	70.5	162.1
Br2	45.5	70.5	80	175.8	46.5	50.5	70	150.5	49.5	59	71.5	144.4
Br3	42.5	67.5	85	200	42	46.5	65	154.8	42	56	69	164.3
Br4	41	68.5	90	219.5	41	46.5	67.5	164.6	43	57.5	70	162.8
Br5	42	68.5	87.5	208.3	41	47	66	161.0	43	57.5	70	162.8

Mitoc v. (Orhei)												
M1	47	71.5	90	191.5	42.5	48,5	70	164.7	43	61.5	83	193.0
M2	46	69	90	195.7	44.5	52	71	159.6	41.5	60.5	82	197.6
M3	42	65	87.5	208.3	42	48.5	69.5	165.5	40	49	67	167.5
M4	44	70	90	204.6	42	48.5	70	166.7	41	60	79	192.7
M5	43.5	68	87.5	201.2	41	46.5	70	170.7	34	43	59.5	175.0
Chisinau (Capriana v.)												
ChC1	40	63.5	82	205.2	42.5	46.5	66.5	156.5	38	53	60	157.9
ChC2	39	66.5	90	230.8	41.0	47	68.0	165.9	40	44	61.5	153.8
ChC3	44.5	66	85	191.0	42	48	67	159.5	42	58.5	67	159.5
ChC4	44.5	68	90	202.3	43	47.5	65	151.2	42.5	58.5	78	183.5
ChC5	42.5	65.5	85	200.0	42	45	65	154.8	41	58	67.5	164.6
Chisinau (Viara v.)												
ChV1	43	66.5	83.5	194.2	45	50.5	71.5	158.9	40.5	51.5	66.5	164.2
ChV2	43	67	84	195.4	41.5	47	65	156.6	40.5	61	74	182.7
ChV3	39.5	56.5	71,5	181.0	42.5	48	66.5	156.5	40	58	73.5	183.8
ChV4	44	67.5	85	193.2	41	47	62	151.2	35	48.5	62.5	178.6
ChV5	45	65	82	182.2	40.5	46	64	158.0	40.5	58	64	158.0
Bacioi v. (Ialoveni)												
B1	43.5	68	87.5	201.2	46.5	50	71	152.7	43.5	61	79	181.6
B2	44	68.5	90	204.6	44	48.5	68.5	155.7	41.5	60	75	180.7
B3	42	66	87.5	208.3	40.5	45.5	66	163.0	41	57	79.5	193.9
B4	42	66	90	214.3	40.5	47	62	153.1	42	60	73	173.8
B5	40.5	64.5	83.5	206.2	42.5	47.5	70	164.7	40	57	67	167.5
Leova sity												
L1	43	67	87.5	203.5	44.5	49.5	69	155.1	41	58.5	78.5	191.5
L2	43	66.5	87.5	203.5	46.5	50.5	70	150.5	42.5	59.5	75	176.5
L3	40.5	68	87.5	216.1	43	49	69,5	161.6	40.5	57.5	76	187.7
L4	43.5	68.5	87.5	201.2	45	47.5	68	151.1	43.5	58.5	77	177.0
L5	45	74.5	90	200.0	47	51.5	71.5	152.1	45	60	70	155.6
Gotesti v. (Cantemir)												
G1	39	52.5	71	182.1	38.5	42.5	57	148.1	38	43.5	59	155.3
G2	39	59	75.5	193.6	40	44	63.5	158.8	38.5	44.5	57	148.1
G3	38	61.5	66	173.7	39	41	56.5	144.9	35	43.5	59.5	170.0
G4	42.5	58.5	73.5	172.9	38	41.5	57	150.0	37.5	42	59	157.3
G5	38.5	53.5	74.5	193.5	36	46.5	57.5	159.7	37.5	46	58	154.7
Doina v. (Cahul)												
D1	34	54	73	214.7	29	39,5	56.5	194.8	28.5	29	39	136.8
D2	34	55	71	208.8	35	46	60	171.4	30	33	46.5	155.0
D3	24	32	37.5	156.3	23.5	27.5	32.5	138.3	25.5	40.5	34	133.3
D4	35	51	67	191.4	34	41	55	161.8	28.5	35	38.5	135.1
D5	24	31.5	36.5	152.1	31	32.5	34	109.7	29	31	36	124.1

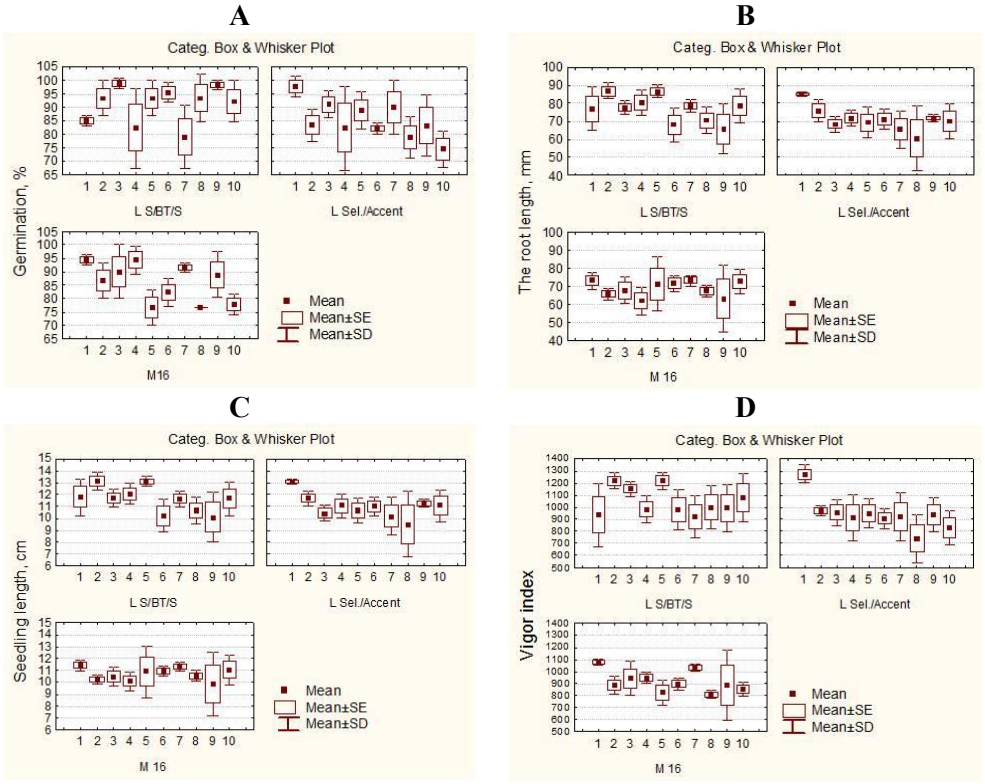
I – colony diameter on day 4; II – diameter of the colony on day 6; III – diameter of the colony on day 8; III/I – colony growth (%) at day 8 compared to day 4.

So, research of 40 isolates of *F. oxysporum* var. *orthoceras* extracted from common winter wheat plants with signs of root rot showed a wide diversity of them based on mycelium color and radial growth speed, which denotes the existence of a pronounced genetic polymorphism of these disease agents.

Biometric analysis data demonstrated that the characters of the wheat genotypes under study, reacted differently to the treatment of grains with culture filtrates (CF) of *F. oxysporum* var. *orthoceras* isolates, their sensitivity depending on the genotype of the plant, the fungus isolate and temperature (Fig. 4).

At the temperature 22–23°C, the difference in the average of the 9 *F. oxysporum* var. *orthoceras* isolates compared to the control was in the case of *germination*: +6.8%, -14.4%, -9.4%; *radicle length:* -0.1%, -18.6%, -11.4%; *seedling length*: +0.2%, -17.6%, -7.0%; *vigor index*: +13.5%, -29.3%, -16.7%, respectively, for the genotypes L S/BT/S, L Sel./Accent, Moldova 16.

At the temperature 22-23/8-9/22-23°C, the difference in the average of the 9 *F. oxysporum* isolates compared to the control was in the case of *germination*: –5.4%, -6.4%, -3.7%; *radicle length:* -7.0%, -7.5%, +2.0%; *seedling length*: -5.7%, –7.7%, +2.8%; *vigor index*: -9.1%, -14.3%, -2.7%, respectively, for the genotypes L S/BT/S, L Sel./Accent, Moldova 16.



22-23°C

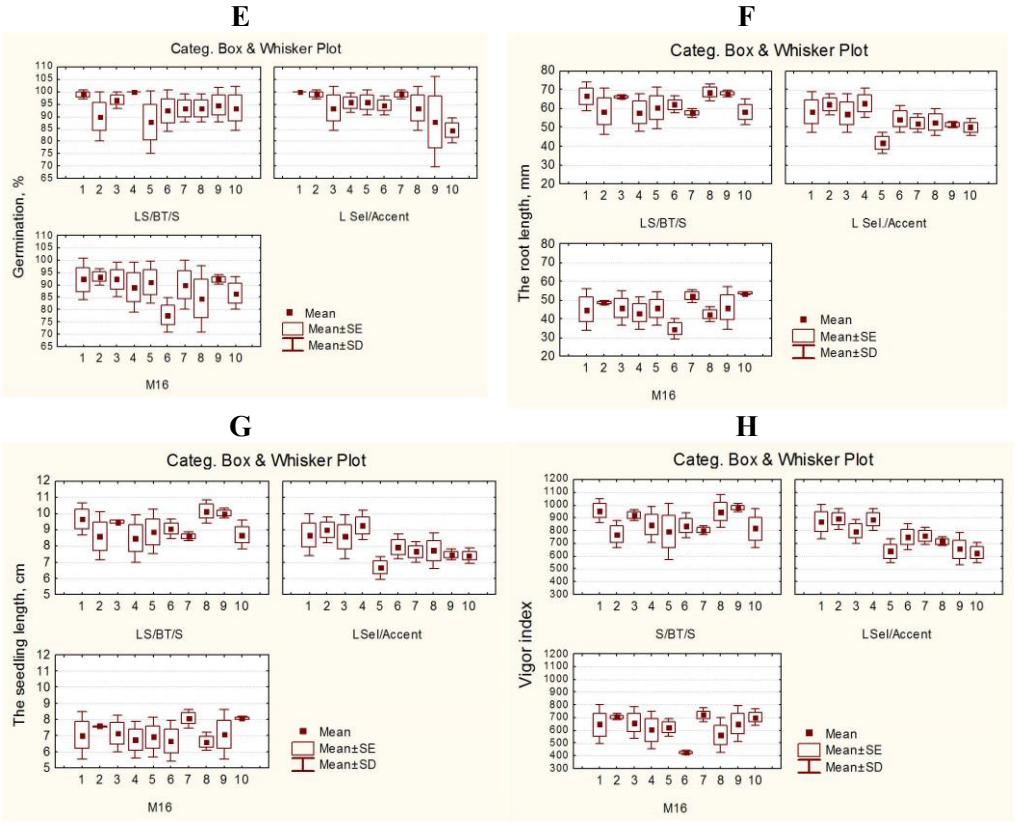


Figure 4. Influence of the *F. oxysporum* var. *orthoceras* culture filtrates and temperature on growth characteristics of common winter wheat plants.
Horizontally: 1 – H_2O, 2 – Br1, 3 – Br2, 4 – Br3, 5 – ChV1, 6 – ChV3, 7 – ChV5, 8 – L2, 9 – L3, 10 – L5.

It is worth mentioning, at the temperature 22–23/8–9/22–23°C the genotype L S/BT/S differed from the other two in that the values of growth and development characters in the 9 variants with FC were practically at the level of the control variant (H_2O), and in the case of germination, FC that stimulate the character were also recorded. This denotes the resistance of the genotype to the action of all *F. oxysporum* isolates. At the thermal alternation 22–23/8–9/ 22–23°C, as can be seen from the data obtained, the M16 genotype demonstrated better results.

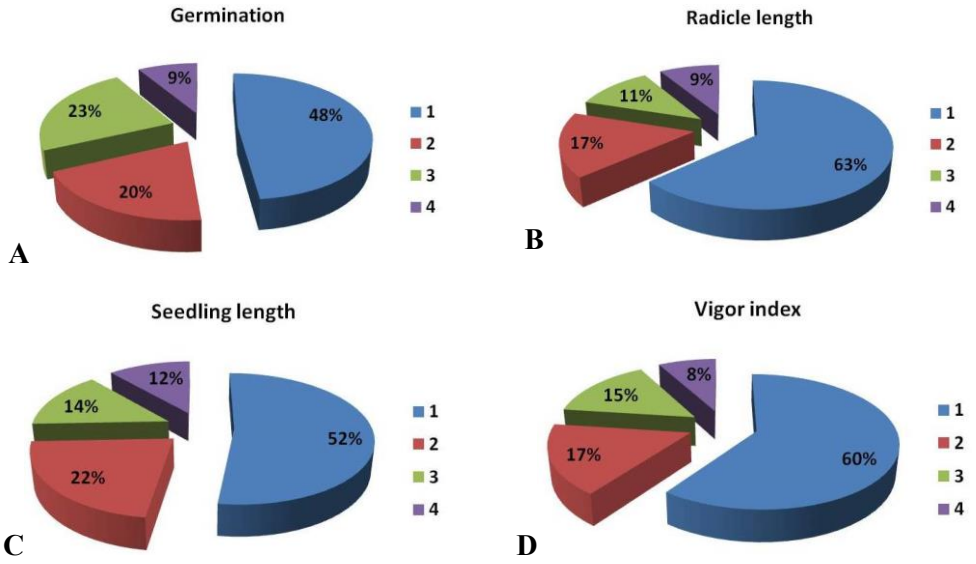
Analyzing the action of the isolates on the vigor index, it can be observed that at the optimal temperature only a decrease in the index was recorded in the

genotypes LSel./Accent and M16, at L S/BT/S only stimulation was manifested (Br1, Br2, ChV1) and lack of effect in the other variants. At thermal alternation a decrease in the vigor index was found in LS/BT/S (ChV5), L Sel./Accent (ChV1, ChV3, ChV5, L2, L3, L5), M16 (ChV3). The data obtained did not find any dependencies between the adaptation capacity of *F. oxysporum* isolates at 9 and 35°C and that of virulence.

Factorial analysis of the weight of plant genotype, *F. oxysporum* isolate, *genotype* × *isolate* interactions in the source of variation of the characters under study returned the genotype that recorded: 48, 63, 51, 60%, respectively, of germination, radicle length, seedling length, vigor index at the temperature **22-23°C**. The weight of the isolate factor constituted: 20, 17, 23, 17%, and of genotype × isolate interaction – 23, 11, 14, 15%, respectively, of germination, radicle length, seedling length, vigor index (Fig. 5).

At thermal alternation 22–23/8–9/22–23°C, the share of the wheat genotype recorded 59, 91, 90, 88%, and of the isolate – 17, 3, 2, 5% in the germination, radicle length, seedling length, vigor index, source of the variation, respective (Fig. 5).

The share of *genotype – isolate* interactions recorded 11 … 23% and 2 …11%, respectively, the optimum temperature and temperature alternation (Fig. 5).



22-23°C

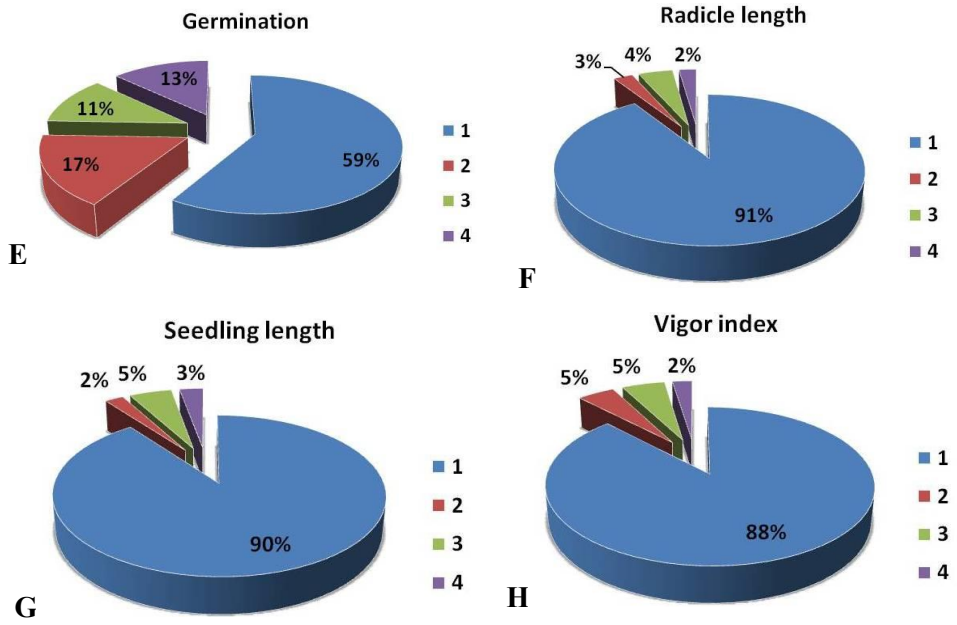


Figure 5. Share of plant genotype factors (1), isolate (2), genotype × isolate interactions (3), random factors (4) in the source of variation in growth and development traits of common wheat at different thermal conditions.

Contribution of *genotype – isolate* relationships in the source of variation of wheat growth and development traits constituted at the thermal alternance **22–23/8–9/22–23°C** the highest values were recorded for the genotype factor: 58.9 ... 90.9%. The role of the isolate factor recorded 2.23 ... 16.72%, the lowest values belonging to seedling length, and the highest – to germination. The share of genotype – isolate interactions recorded 3.66 ... 11.49% (Fig. 5).

Cluster analysis using the dendrogram construction method demonstrated that the classification of isolates based on the effect on germination (%), radicle length (mm), seedling length (cm), and vigor index is different for the 3 genotypes, which denotes that the similarity of isolates according to virulence capacity depends on the genotype of the wheat plant (Fig. 6).

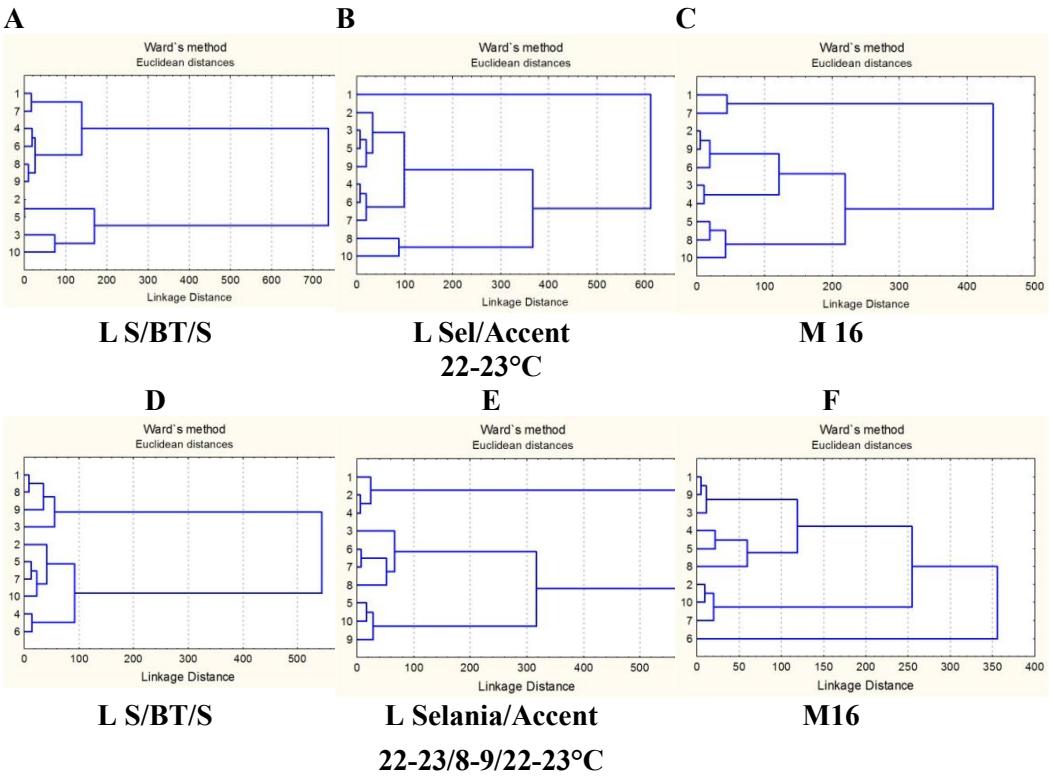


Figure 6. Dendrogram of distribution of *F. oxysporum* var. *orthoceras* isolates based on their action on the growth and development organs of common wheat plants.

From the dendrograms of the distribution of isolates based on the reaction of the characters under study, it is observed that the cluster profile is different due to the differences in similarity of the *F. oxysporum* isolates. Thus, the hypothesis of the Kosman *et al.* (2019) authors is confirmed that the ***isolate*** represents an ***Operational Unit*** depended on its interaction with the host plant.

CONCLUSIONS

The *in vitro* research of 40 isolates of *F. oxysporum* var. *orthoceras* extracted from common winter wheat plants with signs of root rot showed a wide diversity of them based on mycelium color, morphology and radial growth speed in different thermal conditions, which denotes the existence of a pronounced genetic polymorphism of these disease agents.

The different profile of the distribution dendrograms of *F. oxysporum* var. *orthoceras* isolates based on the influence on the growth and development organs of the 3 wheat genotypes under study reveals that the virulence phenotype of the isolates depends on the specific interaction with the host plant, which reflects on the cluster organization of the pathogens.

Factorial analysis of the contribution of the phytopathosystem components in the source of variation in wheat growth and development traits demonstrated that the role of ***the isolate*** varied within the limits of 17–22% at a temperature of 22–23°C and 2 ... 17% – at thermal alternation, which denotes that the variability of the pathogen's virulence phenotype depends greatly on thermal conditions.

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